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That I am knowledgeable in the English and German languages;

That I can translate from German to English;

That the English translation attached hereto is a true and complete translation of the German language International Application PCT/EP98/04726.

That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Medium for Producing and/or Treating Alcoholic Beverages, Especially Wine or Sparkling Wine, as Well as Its Applications

Description

The invention refers to a medium for producing and/or treating alcoholic beverages, especially wine or sparkling wine, in accordance with the generic meaning of the principal claim as well as its applications.

Various species of microorganisms, especially yeast used for alcohol fermentation, are employed in the production of alcoholic beverages, especially of wine or sparkling wine. To optimize the results, other species of microorganisms and enzymes are added to the product or its preliminary stages. Thus, for example, lactic acid bacteria serve to break down malic acid and pectins accelerate must clarification.

The process of alcohol fermentation can be interrupted by such processes as rapid cooling down, addition of sulfur dioxide, or filtration. These processes for inactivating the yeast are, however, costly, only imprecisely controllable, and can impair the product's quality. Thus, a time-delayed use of various yeast species turns out to be costly.

After handling, for example, a wine with lactic acid bacteria to reduce the acid content, the added microorganisms have to be separated, which takes place with the relatively small lactic acid bacteria via membrane filtration. However, in this connection there is not always a guarantee of complete removal of microorganisms. Lactic acid bacteria remaining in the wine can transform glucose into acetic acid, thus ruining the wine.

Enzymes such as proteases to break down peptides and proteins are added to the product or its preliminary stages in liquid form. The inactivation of enzymes as a rule takes place by heating, whereby spoilage to the product can result, as well as excluding a recycling of in part expensive enzymes.

There are mediums known in sparkling wine production for bottle fermentation that consist of yeast's immobilized in alginate beads (G. Troost, et al., Sekt, Schaumwein, Perlwein [Champaigne, Sparkling Wine, Perlant Wine] Stuttgart 1995 and DE 39 08 997 A1). With this, the time-consuming manual riddling of the fine yeast deposits could be replaced by quickly sinking the alginate beads in the champagne bottle. The disadvantage of such mediums, in which the beads are not surrounded by a cell-free membrane cartridge, however, is that they do not show any high mechanical stability, and cannot sufficiently prevent growth of especially the relatively smaller microorganisms, whereby the microorganisms can remain in the product after separation of the beads. A multiple use of such medium can only be realized with difficulty. Furthermore, enzymes are usually not immobilizable because of their small quantity in such alginate beads.

US 4,996.150 describes a process for the microencapsulation of biocatalysers, preferably yeast, as well as their use in the continuous production of ethanol. The biocatalysers are contained in a matrix of an anionic polysaccharide and a cationic polymer. Here, too, the microcapsules do not display any cell-free membrane cartridge, so that yeast growth cannot be sufficiently prevented. Additionally, it is hardly possible to immobilize the smaller biocatalyzers, such as enzymes, with sufficient surety.

US 4,659,662 describes a process for producing alcoholic beverages or bioalcohol by using yeast-containing microcapsules. The yeast can be embedded in a matrix material that is additionally surrounded by a membrane cartridge, one that, however, is the same as the matrix material. Yeasts immobilized in calcium alginate were named as an example. The membrane cartridge, however, consists of just a single layer and is, moreover, infiltrated with cells, that is, not cell-free. Thus, growth of the yeasts cannot be sufficiently prevented and a sufficient immobilization of enzymes with long-term stability is hardly possible.

DE 34 32 923 C2 concerns biocatalyzers with immobilized cells that are surrounded by a single-layer, cell-free membrane cartridge. The membrane cartridge, which

surrounds the inner capsule and is not cell permeable, can consist of a interlinking ionic or covalent gel.

Champagne production will be used as an applications example. The membrane cartridge consists preferably of the same substance as the matrix material in the inner capsule. The interlinkingmedium, for example calcium ions, containing a surplus of the beads, here out of calcium malginate, is again put into a cell-free substance, here an alginate solution, that forms the membrane cartridge. The disadvantage with this is that liquefaction of the capsule's contents is not possible without dissolving the membrane cartridge. Especially with enzymes, however, a liquifacted inner capsule facilitates the preservation of natural conformity and thus the enzyme activities. Beyond this, such membrane cartridges hardly allow for attaining standards such as targeted permeability and a sufficiently high mechanical stability.

The purpose of the invention is to make available a medium for producing and/or treating alcoholic beverages, especially wine or sparkling wine, according to the generic term of the primary claim, in which the cells or enzymes are permanently immobilized, in which the permeability and the mechanical stability of the membrane cartridge is adjustable, and in which the contents of the microcapsules can be liquefied.

The invention has the further purpose of demonstrating applications relating to the medium according to the invention.

The purpose will be evidenced through a medium with the characteristics of claim 1 as well as through the applications according to claims 20 and 21, whereby the sub-claims address the advantageous aspects of the invention.

The species of microorganisms and/or enzymes used in the production and/or treating of alcoholic beverages, especially wine or sparkling wine, are immobilized in that they are contained in the inner capsule, and that a membrane cartridge completely surrounds the inner capsule. The membrane cartridge for these microorganisms or

enzymes is not permeable, thus preventing the escape of the microorganisms or enzymes. In order to ensure a substance conversion, the membrane cartridge for the substances to be converted (output product) is permeable, also included in this are the nutrients necessary for the microorganisms, for example, glucose; the membrane cartridge is also permeable to at least a part of the produced or converted substances (products), alcohol and carbon dioxide, for example. The requirements of permeability and mechanical stability were fulfilled by the membrane cartridge that has at least two layers, radially arranged on top of each other, whereby each layer completely encloses all of the radially arranged layers beneath it. The advantage here is that the individual layers are bound together ironically and/or covalently.

Only this multi-layered construction allows the permanent immobilization of cells or enzymes for the production and/or treating of alcoholic beverages, such as wine or sparkling wine. So, for instance, this effectively prevents an outgrowth of yeast's and with this an accompanying damage of the microcapsules. With the high mechanical stability, the microcapsules can be used in larger bioreactors without squashing or popping the microcapsules. This increased stability also enables the inner capsule to liquefy, without the microcapsules becoming too mechanically instable in the process. Additionally, the permeability of the membrane cartridge can be selectively adjusted by a suitable selection of at least two layers, which is of decisive importance for immobilizing the enzymes.

Preferably, not only the outer layers, but also the innermost layer of the membrane capsule evidence none of the cells or enzymes contained in the inner part of the microcapsules.

Preferably, at least two layers of the membrane cartridge consist of different substances. Thus, for example, an outer layer (support layer) can consist of a substance that ensures a high level of mechanical stability, while an inner layer (control layer) consists of a substance that enables a selective adjustment of this layer's permeability and with that the membrane cartridge's permeability.

After an advantageously executed form, the cells or enzymes contained in the inner microcapsules are embedded in a matrix. This matrix can be constructed from an alginate combination with a polyvalent cation, for example, calcium, strontium, barium, aluminum and/or iron.

According to another advantageously executed form, the cells or enzymes in the inner microcapsules freely move about in a fluid, which is especially advantageous for maintaining the enzyme's natural conformation. With this, the cells' or enzymes' natural activity is maintained despite being immobilized. If, by means of an interlinking medium, the microcapsules are produced by the precipitation of beads of a solution containing cells or enzymes, the inner capsule can again be liquefied after the membrane cartridge's layers are fixed. If, for example, the matrix substance in the inner capsule consists of calcium alginate, then the polyvalent metallic cation can be exchanged for a monovalent cation, for example, natrium or calcium, in order to again liquefy the inner microcapsule. It is thus preferable that at least one layer of membrane capsule consists of one of the different substances that embed the cells or enzymes and that constitute the matrix. As far as known microcapsules are concerned, where the single layer membrane cartridge consists of the same substance as the matrix in the inner capsule, this liquefaction of the inner capsule is not possible, since the membrane cartridge would in this case also dissolve.

In the case of an interlinkable substance using polyvalent cations, a further advantage of exchanging the polyvalent cations for monovalent cations is that polyvalent cations, such as calcium, are as a rule undesirable in wine production. Thus, for example, a matrix of calcium alginate can become liquefied by introducing the microcapsules into a watery solution containing natrium citrate, which creates an exchange of calcium ions for natrium ions. These microcapsules, such as those used in wine production for example, preferably will bond polyvalent ions from the substrate to be converted, such as grape juice, which has an advantageous effect. If these microcapsules' matrix again shows a too-high content of polyvalent cations,

then the matrix can be regenerated again through treating it with a solution containing monovalent cations.

Microcapsules with a membrane cartridge out of one layer as well as out of multiple layers are known in the medical field to immobilize cells or enzymes. Thus, Langerhansian cells are encased in a microcapsule, with the membrane cartridge being constructed from a layer consisting of alginate and poly-L-lysine. (F. Lim et al., Science, 210 (1980) 908-910). EP-A-681834 described microcapsules, with the membrane cartridge being constructed of numerous layers, for introduction into living organism tissue.

The requirements of such microcapsules are tissue tolerance, a low level of immune reaction, and ability to introduce them into living organism tissue.

By optimizing microcapsule construction with a multi-layered membrane cartridge with a view to the production or treating of alcoholic beverages, suitable microorganisms, such as yeast used in alcohol fermentation or lactic acid bacteria, and/or enzymes, can be immobilized advantageously. Particularly in the case of immobilizing yeast for alcohol fermentation, stability has to be ensured, despite the production of carbon dioxide and a rapid growth of yeast cells. This is achieved through microcapsules with membrane cartridges that have at least two layers arranged radially above each other.

The medium according to the invention has an advantage over the already familiar mediums [used] in the production of alcoholic beverages in that the encapsulated cells or enzymes are permanently immobilized and, because of the simple operations process, can be easily measured out and added and can also be simply, quickly, and completely removed from the product. This allows for a specific influence of the individual production steps without impairing product quality. Additionally, microcapsules containing cells or enzymes can be reused, which leads to cost savings where expensive enzymes are used. Further, a good substance exchange, especially the gas exchange in alcohol fermentation, is ensured because of the hollow spaces

present between the capsules even where sedimentary microcapsules are involved, as well as because of the microcapsules' easy mobility in a fluid substance.

It can be advantageous to make the membrane capsule impermeable to those active substances and/or microorganisms situated outside the microcapsule that could impair the activity of the cells or enzymes contained in the inner capsule. Thus, some species of yeast used in wine production produce toxins that are harmful to other species of microorganisms. By using microcapsules having a membrane cartridge that is not permeable by such toxins, such microorganisms or enzymes can be introduced together, for example, into wine production.

After a preferred implementation method, the microcapsules contain as microorganisms at least one of the yeast species employed in the wine production process.

After a further preferred implementation method, there is in the microcapsules at least one species of the lactic acid bacteria that are used in biological acid breakdown in wine production.

Processes to deacidify wine by using cells of the Leiconostoc oenos species immobilized in calcium alginate are known from US 4,380,552. These beads, however, have no membrane cartridge, thus not ensuring sufficient stability and an outgrowth of cells.

Advantageously suited as a method of producing or treating alcoholic beverages are microcapsules that contained enzymes such as pectases, glycosides, β-glucosidases, proteases, and/or glucose-fructose-isomerases used especially in wine production.

It can be advantageous to immobilize cells as well as one or more enzymes in a microcapsule. Firstly, this can make implementation easier, and secondly, this can especially increase productivity if a product of the microorganism or enzyme is

further converted by the other microorganisms or enzymes contained in the microcapsule. Thus, species of yeast and their treatment substances such as the yeast cell wall preparations and/or glucose-fructose-isomerases used in wine production can be advantageously immobilized together in a microcapsule. Aside from an increase in activity of the yeast used, the quantities of, in part, expensive treatment substances to be used can be reduced.

Activity of the immobilized cells or enzyme-increasing substances can also be encapsulated in a microcapsule. It is thus, for example, known that the activity of lactic acid bacteria decreases with an increase in acid content. By including a cation exchanger in a microcapsule containing lactic acid bacteria, the pH-level can be increased at the site of lactic acid bacteria through an exchange of hydrogen ions for, for example, potassium ions, thus increasing the activity of lactic acid bacteria. Further examples of activity-increasing substances are vitamins, such a vitamin B_1, or growth-promoting proteins.

Preferably, at least one layer of the membrane cartridge will consist of at least one polymer. Suitable as a polymer would preferably be a polyelectrolyte complex that consists of at least one polycation and one polyanion. Suitable polyanions are, for example, polyacrylic acid, polymethacrylic acid, polyvinylsulfonic acid, polyvinylphosponic acid, alginate acid, cellulose derivatives, especially carboxylmethyl cellulose or cellulose-sulfuric acid ester, shellac or shellac components such as aleuritin acid or shellol acid. Suitable polycations are, for example, polyethylenimine, polydimethyl dialylammonium, poly-L-lysin or chitosan.

The polyanions or polycations will preferably have a mid-polymerization level of above 100, preferably from 100 to 15,000. For the specific adjustment of the membrane cartridge's permeability, the polymers preferably used for the layer that determines the permeability (regulating layer) are polymers with a narrow distribution of molecular mass, for example, synthetic polyelctrolytic complexes from polyacrylic acid or polymethacrylic acid with polyethylnimine. Thus, the permeability of a small

protein having a quantity of about 60 kD is attained through a regulating layer of polyethylenimine (PEI) (molecular mass 1,000,000) and polyacrylic acid (PAS).

Table 1 shows the portion of protein diffused from the microcapsule in connection with the level of PAS polymerization after 60 hours of riddling.

Regulating Layer	Molecular Mass of NaPAS	Level of Polymerization	Permeability After 60 Hours
PEI/PAS	20,000	215	8.4%
PEI/PAS	60,000	645	18.9%
PEI/PAS	170,000	1828	93.1%

Table 1. Permeability of the regulating layer for a 60 kD protein in connection with the molecular mass of a polyanion as natrium-polycrylate (NaPAS).

As seen in Table 1, the permeability of the regulating layer is determined by the polyions, in this case a polyanion polyacrylic acid that has a small level of polymerization, while the counter ions, in this case the polycation polyethylenimine with a high level of polymerization, form the framework. Where there is the same counter ion, the polyelectrolytic complex with a poly ion with a high level of polymerization has a greater strength than those with a poly ion with a low level of polymerization. As framework-forming counter ions that take up the polyions with a lower level of polymerization, polyions with a polymerization level of 50,000 are preferred.

To immobilize the enzymes, at least one regulating layer with a polymerization level of the polyanions or polycations of 100 to 1,000 is preferred, whereby the framework-forming counter ions, polycations or polyanions have higher polymerization levels. To immobilize microorganisms such as yeast, in contrast, higher polymerization

levels are sufficient; here polymerization levels of 1,000 to 15,000 of polyanions or polycations in the regulating layers are preferred, whereby here, too, the framework-forming counter ions, polycations or polyanions, have a higher level of polymerization. Polyions with high polymerization levels have the advantage of greater strength in the relevant layer.

For the strength-determining layer (support layer), polyelectrolyte complexes of synthetic polycations or polyanions with high polymerization levels of over 10,000 are preferably used. Natural polycations and anions such as alginate acid and chitosan or cellulose derivatives can also be used advantageously; with these, a wide molecular mass distribution does not have a disruptive effect.

Table 2 shows examples for substances in the membrane cartridge layers, whereby each first row lists whether this structure is preferably suited for immobilization of yeast and/or enzymes. The core can have an alginate for immobilization that can be liquefied after the layers have been put in. In each Table 2 column, the layer structure from inner to outer is given, With a larger number of layers, for example 4 or more layers, a still greater membrane cartridge stability can be achieved.

	Yeasts	Yeasts	Yeasts and/or Enzymes	Yeasts and/or Enzymes	Enzymes	Enzymes
1. Layer	PEI/CMC	PEI/CMC	Chit/PAS	Chit/PAS	PEI/PAS	PEI/PAS
2. Layer	PEI/CMC	PEI/CMC	Chit/CMC	Chit/CMC	PEI/CMC	PEI/CMC
3. Layer		PEI/CMC		Chit/CMC		PEI/Alg

Table 2. Examples of the Membrane Cartridge's Layer Structure with Immobilized Yeasts and/or Enzymes (Alg = Alginate, Chit = Chitosan, CMC = Carboxymethyl cellulose, PAS = Polyacrylic acid, PEI = Polyethylimine).

In addition, natural rubber, polystyrene and/or polymethylmethacrylate or a mixture thereof with one or more polyelectrolyte complexes are suitable as polymers for building a membrane cartridge layer.

The invention also concerns two applications for the medium according to the invention. Thus the medium can be used in beer production. Here, microcapsules are to be used with cells from one or more yeast species used in beer production. Likewise, the medium can be used for producing low-molecular alcohol such as methanol and/or ethanol, whereby microcapsules with yeasts suitable for high-yield alcohol production are used.

To produce and/or treat wine, for example, using the medium according to the invention, the microcapsules are to be put into a grape, berry and/or other fruit juice, apple juice, for example, or into a wine. The microcapsules stay in the solution until partial or complete conversion, such as alcohol fermentation, has taken place. Finally, the microcapsules are removed from the solution.

As opposed to already-known mediums in which the microorganisms or enzymes used have to be filtered off, this medium has the decisive advantage of allowing the cells or enzymes used in the production or treatment process to be simply, quickly, and completely removed in the last step of the process. With this, the time that microorganisms or enzymes remain in the solution can be precisely adjusted.

Because of the microcapsules' size, diameters of one-half or less millimeters are preferred the microcapsules can be simply and completely removed from the solution. Mechanical processes are appropriate here, for example by using a sieve, or decanting the liquid remaining after prior sedimentation of the microcapsules. In these removal processes, the microcapsules are not destroyed, so that they can be reused if need be after being stored in the interim in a nutritive solution. Especially in the case of expensive enzymes, production costs can be reduced.

Advantageously, at least in the area of microcapsules, the solution is set to just the temperature that makes the cells or enzymes optimally active, whereby a possible influence in the product quality is to be considered.

Various microorganisms or enzymes can be used in microcapsules simultaneously and/or time-delayed. In the case of time-delayed usage, the various microcapsules can be successively introduced into the solution and removed together, or the processing steps of introduction, time spent in the solution, and removal are successively run through multiple times with the same solution by using various cells and enzymes immobilized in the microcapsules. The use of different microorganisms or enzymes can thus be used selectively to increase the complexity of, for example, a wine.

A fluidized bed reactor, which contains the medium according to the invention, can be used as a bioreactor for production of low-molecular alcohol, especially ethanol, or alcoholic beverages, especially wine or sparkling wine. Suitable reactor types are also listed in Lüders, "Technologie mit immobilisierten Hefen" [Technology with Immobilized Yeasts], Brauwelt 1994, 57.

The bioreacter can also have at least one tube that contains the microcapsules. The two tube openings are advantageously sealed with sieves that retain the microcapsules.

This precipitates a separation of the microcapsules from the converted solution. The preferable diameter of suitable tubes is in the area of one or more centimeters.

The liquid to be converted is guided through the tubes, whereby numerous tubes can be connected parallel and/or serially to another. In the case of a simultaneous use of various microorganisms or enzymes immobilized in microcapsules, it is preferable that tubes with the same content be joined parallel to each other, whereby groups of tubes with different contents joined parallel to each other are joined together serially.

It can be advantageous to join together individual tubes serially so that the solution flows through the tubes in succession.

To optimize the activity of the microorganisms or enzymes used, it is advantageous to set the temperature of the inner part of the tubes, that the microcapsules and their surrounding solution. Here, it is advantageous for a single tube or a group of tubes to have a shared temperature-settable sleeve. The activity of the cells or enzymes can be specifically lowered by cooling the microcapsules enclosed in the tubes.

In an installation for the production of low-molecular alcohol, especially ethanol, that have at least one bioreactor, the glucose-containing liquid to be fermented goes from the storage tank into the mixer. There it is mixed with the backflow from the still and then put into the microcapsule-filled bioreactor, where the actual conversion of carbohydrates into alcohol takes place. The alcohol is then separated in the heating container and in the distillation column and gathered in the collection tank for the primary product. The separation of alcohol from the remaining liquid occurs by using the different boiling points. The remaining liquid from the still can, after cooling down in the heat exchanger, again be enriched in the mixer with fresh liquid and go renewed into the bioreactor. In order to avoid an excessive thinning of the glucose in the circulating liquid, a part of the watery portion is regularly removed and gathered as a by-product in a collection tank.

An installation for the production of alcoholic beverages has a greatly simplified structure. The liquid to be fermented is taken from a storage tank and put into an bioreactor according to the invention, where the liquid sits for a period of time or, for example, circulates through a tube reactor. The alcohol-containing product is routed from the bioreactor into a collection tank after the time required for a partial or complete conversion.

Further details of the installations can be found in the following descriptive section in which, with the drawing, an example of implementation is described in more detail. The attached figure shows the schematic structure of a continuous installation for

alcohol production. The liquid to be fermented is taken from a storage tank (1) and put into a mixer (2), where it mixes together with a liquid backflowing from the still (4). This mixture is converted into alcohol in the bioreactor (3) from the yeast immobilized in the medium according to the invention. The alcohol-containing fluid is put into a still (4). From there, an alcohol-containing gas phase is routed into a distillation column (5). There, the different boiling points of alcohol in contrast to water are used to enrich the alcohol that is gathered in a collection tank for the primary product (6). The alcohol-lacking portion of the pre-fermented liquid is taken out of the still and cooled in a heat exchanger (8) and routed back into the mixer (2). In order for the liquid in circulation to contain a minimum level of the substances to be converted, especially glucose, a part of the watery portion is regularly removed from the still (4) and gathered as a by-product in a collection tank (7).

Example of Microcapsule Production:

5 g ~~natrium~~ sodium-alginate (Kelco Co., Hamburg) are dissolved in 700 ml water. Then 70 g of dry yeast was subsequently stirred into this solution (Oenoform, Erbslöh Co., Geisenheim). This suspension was dropped into a 0.6% solution of calcium chloride. After a few minutes of curing time, the beads containing the yeast cells in a calcium-alginate matrix were first washed with water and subsequently with a watery 0.05% solution of polyethylenimine (mid-range molecular mass 1 mil, Fluka Co.) and was afterwards washed with a watery 0.06% of carboxymethyl cellulose (mid-range viscosity, Fluka Co.). Subsequently, the microcapsules thus obtained were washed with water and once again put into the polyethylynimine and carboxymethyl cellulose solution. After being cleaned with water, the microcapsules were stored in water. The microcapsules have a two-layered membrane cartridge, whereby each layer consists of the polyelectrolyte complex of polyethylynimine and carboxymethyl cellulose. Because the calcium alginate beads showing cells were produced first and because the layers were subsequently applied to the membrane cartridge, the membrane cartridge layers showed no yeast cells that could grow out of the microcapsules. The yeast thus immobilized showed the same activity as in yeast immobilized in unlayered calcium-alginate beads.